

# Histamine Receptors on Bovine Peripheral Blood Lymphocytes

Hisashi INOKUMA, Roy L. KERLIN<sup>1)</sup>, David H. KEMP<sup>1)</sup>, and Peter WILLADSEN<sup>1)</sup>

Livestock Industry Bureau, Ministry of Agriculture, Forestry and Fisheries, 1-2-1 Kasumigaseki, Tokyo 100, Japan and <sup>1)</sup>CSIRO, Division of Tropical Animal Production, Long Pocket Laboratories, Private Bag No. 3, Indooroopilly, Queensland, Australia

(Received 17 June 1993/Accepted 6 September 1993)

**ABSTRACT.** Histamine receptors on bovine peripheral blood lymphocytes (PBL) were detected by three different methods: a rosetting technique, binding to histamine-bearing Sepharose beads and immunofluorescence staining. The rosetting technique used histamine-rabbit serum albumin (H-RSA) conjugated to bovine red blood cells to detect histamine receptors and this showed that 10.8% of bovine PBL were positive. A method using H-RSA conjugate coupled Sepharose beads also detected histamine receptor bearing PBL but was not quantitative. The indirect immunofluorescence method, by which the subpopulation of histamine receptor bearing lymphocytes can be easily double stained to concurrently identify the B cell marker, revealed that PBL, the B cell and T cell fraction of bovine PBL contained 18.4, 52.8 and 9.3% histamine receptor bearing cells, respectively. This method was found to be more stable and more easily quantifiable than the other two methods. Blocking tests using the histamine H1 receptor antagonist diphenhydramine and the histamine H2 receptor antagonist cimetidine suggested that bovine PBL have both H1 and H2 receptors on their surfaces. Addition of histamine into cultures of PBL at the concentration range  $10^{-6}$  to  $10^{-3}$  M suppressed the response of PBL to the mitogen phytohemagglutinin. The histamine induced suppression of mitogenesis could be reduced partially by the H2 receptor antagonist cimetidine, but not by the H1 antagonist diphenhydramine. It is possible that histamine induced suppression of PBL mitogenesis was mediated by H2 receptors on T cells.—**KEY WORDS:** bovine peripheral blood lymphocyte, histamine receptor, immune suppression, mitogenesis.

— J. Vet. Med. Sci. 56(1): 45–49, 1994

Histamine is able to regulate the immune response in a number of species by binding to histamine receptors on lymphocytes. Several studies have suggested that histamine may both enhance lymphocyte mitogenesis by acting via histamine H1 receptors, and also suppress mitogenesis by acting via histamine H2 receptors [1, 2, 18, 19, 23].

Increased numbers of abnormal functions of histamine receptor bearing lymphocytes are thought to be responsible in part for a variety of pathological conditions in humans such as autoimmune disease [5], atopic dermatitis [4], neoplastic disease [24] and infection with the parasite *Schistosoma mansoni* [7]. Histamine receptors on peripheral blood lymphocytes (PBL) have also been studied in guinea pigs [14, 15], mice [8, 22], jirds [9, 17] and dogs [11, 23] to determine the relationship of histamine to the immunopathogenesis of diseases such as atopic dermatitis in dogs and filariasis in jirds. Although histamine is also one of the important mediators of inflammation and immune reactions in bovine, there is no information available about histamine receptors on bovine lymphocytes.

In the present study, we determined that bovine lymphocytes also bear histamine receptors. We examined the effect of histamine on bovine lymphocyte function *in vitro*, too.

## MATERIALS AND METHODS

**Drugs:** Histamine dihydrochloride, the H1 antagonist diphenhydramine and H2 antagonist cimetidine were purchased from Sigma Chemical Company (St. Louis, MO.). All chemicals were dissolved in RPMI 1640 (Flow

Laboratories, Irvine, UK) and the pH was adjusted to 7.2 with 1N HCl and 1N NaOH. Chemicals were kept at  $-20^{\circ}\text{C}$  until used.

**Preparation of histamine-rabbit serum albumin (H-RSA) conjugate:** The H-RSA conjugate was prepared by the procedure described by Kedar and Bonavida [8]. Histamine dihydrochloride (1.4 g), RSA (200 mg) and 1-ethyl-3-(3-dimethyl-aminopropyl) carbodiimide hydrochloride (ECDI, 1.2 g, Sigma) were dissolved in 20 ml of phosphate-buffered saline (PBS, pH 7.2). After incubation for 1 hr at room temperature with intermittent shaking, the solution was dialyzed at  $4^{\circ}\text{C}$  against 5 liters of PBS which was changed three times over 48 hrs. The control conjugates were prepared in parallel under the same conditions. RSA-ECDI conjugate which was composed of RSA and ECDI, or RSA alone were used as control. These preparation were stored at  $-20^{\circ}\text{C}$  until used.

**Separation of PBL:** Heparinized peripheral blood were collected from three female Hereford cows (18 months old). PBL were obtained from the fresh heparinized blood by density gradient centrifugation with Ficoll-Paque (Pharmacia, Uppsala, Sweden) [12]. PBL were then incubated for 1 hr at  $37^{\circ}\text{C}$  in an humidified atmosphere of 5%  $\text{CO}_2$ : 95% air to remove adherent macrophages or monocytes. After incubation, non-adherent cells were resuspended at  $2 \times 10^7$  cells/ml in complete RPMI 1640 medium containing 10% heat-inactivated foetal calf serum (Flow Laboratories), 2-mercaptoethanol ( $5 \times 10^{-5}$  M), penicillin (100 IU/ml) and streptomycin (20  $\mu\text{g}/\text{ml}$ ).

**Isolation of E-rosette forming T cells:** T cells were isolated from PBL by a modification of the method of Paul

*et al.* [13], by using 2-aminoethylisothiuronium bromide (AET, Sigma). One volume of isolated PBL was mixed with 1 volume of AET treated sheep red blood cells (AET-SRBC, 1% suspension in PBS), and incubated at 37°C for 15 min, followed by centrifugation at 200 g for 5 min. After incubation at 4°C for 1 hr, E-rosette forming T cells were obtained by density gradient centrifugation with Ficoll-Paque. Those cells were treated with a hypertonic Tris ammonium chloride solution (0.17 M Tris pH 7.2, containing 0.75% NH<sub>4</sub>Cl) for 1 min to remove SRBC, then washed twice with PBS and finally suspended at  $2 \times 10^7$  cells/ml in complete RPMI 1640 medium.

#### *Detection of histamine receptors on cells:*

(1) *Rosette assay:* H-RSA conjugate was coupled to bovine red blood cells by the method of Smart and Kay [21]. Briefly, 0.25 ml of bovine red blood cells (50% suspension in PBS), 1.0 ml of freshly prepared ECDI (20 mg/ml) and 2.5 ml of H-RSA conjugate (or RSA-ECDI or RSA alone, 1:200 in PBS or PBS) were mixed into a tube and incubated for 45 min at room temperature with intermittent agitation. The red blood cells were then washed three times with PBS and resuspended at 1% in PBS. Equal volumes of a PBL suspension ( $1 \times 10^7$  cells/ml) and the H-RSA coupled red blood cells were mixed and centrifuged at 200 g for 5 min at room temperature followed by incubation for 15 min on ice. The resultant cell pellets were resuspended by gentle pipetting and mixed with one drop of 0.1% Toluidine blue in PBS to stain lymphocytes. The stained cells were examined under the microscope, counted with a hemocytometer and the percentage of cells which bound three or more red blood cells were determined for 200 lymphocytes counted.

(2) *Sepharose beads method:* H-RSA conjugate coupled to CNBr-activated Sepharose 4B beads (Pharmacia Fine Chemicals, Uppsala, Sweden) was prepared as described by Weinstein *et al.* [22]. The beads were also reacted with RSA-ECDI, RSA alone and PBS as controls. Coupled beads (10  $\mu$ l) were incubated with 50  $\mu$ l of PBL ( $2 \times 10^7$  cells/ml) at 37°C for 10 min. Using one drop of the suspension, 100 beads were examined under the microscope and scored according to degree of binding (0–10, 11–20, more than 20 cells/beads).

(3) *Immunofluorescence method:* A double immunofluorescence method was used to simultaneously detect histamine receptors and surface membrane immunoglobulin on the cells. Cells (PBL or T cells,  $2 \times 10^6$  cells) were washed twice with PBS containing 0.2% sodium azide, and resuspended in 100  $\mu$ l of PBS. The cell suspension was incubated with 50  $\mu$ l of H-RSA (1:200 in PBS) and 50  $\mu$ l of affinity purified rabbit anti-bovine immunoglobulin for 30 min at room temperature. After washing three times, 50  $\mu$ l of affinity purified sheep anti-RSA conjugated with tetramethyl rhodamine isothiocyanate (TRITC, Nordic Immunology, Tiburg, Netherlands) and 50  $\mu$ l of affinity purified sheep anti-rabbit immunoglobulin conjugated with fluorescein isothiocyanate (FITC, Sigma) were added to the cell pellet followed by incubation for 30 min at room temperature.

After washing three times, the cells were examined with a ultraviolet microscope (Zeiss, Germany). Cells binding FITC labelled antibody were visualized using a 485 nm excitation filter and 500 nm barrier filter, while cells binding with TRITC labelled antibody were identified using a 546 nm excitation filter and 600 nm barrier filter.

To confirm the specificity of these methods, RSA-ECDI conjugate, RSA alone or PBS were used instead of the H-RSA conjugate.

*Blocking by H1 and H2 antagonists:* Both H1 and H2 antagonists were adjusted to  $10^{-3}$  M in PBS at pH 7.2 before being used. Histamine (or H-RSA) and either antagonist were added simultaneously in the cell culture or immunofluorescence assay.

*Cell culture:* PBL were dispensed at  $2 \times 10^5$  cells in 50  $\mu$ l per well in triplicate wells into 96 well microculture plates (Disposable Products, Adelaide, Australia). Histamine dihydrochloride was added to wells at final concentration of  $10^{-5}$  to  $10^{-7}$  M. An optimal concentration of phytohemagglutinin (PHA, Sigma, final concentration 10  $\mu$ g/ml) was added to each well before incubating the culture. In some cultures, an H1 or H2 antagonist was also simultaneously added to the wells at the same final concentration as histamine. The final volume of each well was adjusted to 150  $\mu$ l. The plates were incubated for 72 hrs at 37°C in a humidified 5% CO<sub>2</sub> atmosphere.

The blastogenic activity in the cultures was measured using a colorimetric tetrazolium salt assay [10]. The plates were read using a Titertek Multiscan plus ELISA reader (Flow Laboratories) with a wavelength of 600 nm. The optical densities (OD) of unstimulated control wells were subtracted from the OD value of experimental wells.

The suppression of blastogenesis caused by added histamine or antagonists was expressed as a percentage.

% suppression =  $100 \times (\text{OD of PHA stimulated cells} - \text{OD of PHA plus drug stimulated cells}) / (\text{OD of PHA stimulated cells} - \text{OD of control cells})$

## RESULTS

*Detection of histamine receptors on bovine peripheral blood lymphocytes:* We used three different methods to detect the histamine receptors on bovine PBLs. By the rosette method, 10.8% of PBL from three healthy cattle were found to possess histamine receptors. RSA-ECDI, RSA alone or PBS treated red blood cells did not make any detectable rosettes with bovine PBL. Table 1 shows the results of the method using H-RSA conjugate-coupled Sepharose beads. Fourteen percent of H-RSA coupled beads bound more than 20 cells and 19.0% of beads bound 11 to 20 cells. A small number of RSA-ECDI coupled beads (3.0%) bound from 11 to 20 bovine lymphocytes, but RSA alone or PBS coupled beads did not bind bovine lymphocytes. PBL from three healthy cattle showed 18.4% histamine receptor-positive cells by the indirect immunofluorescence method, while no positive cells were detected when RSA alone, ECDI-RSA or PBS were used in the first step instead of H-RSA.

**Blocking of histamine receptors by H1 or H2 antagonists:** The results of an experiment using an H1 or H2 antagonist in the immunofluorescence test for histamine receptors on bovine lymphocytes are shown in Fig. 1. Both H1 and H2 antagonists reduced the percentage of histamine receptor positive cells in PBL in a dose-dependent fashion. There appeared to be little variation in the degree of fluorescence of positive cells.

**Quantitation of histamine receptor bearing B and T cells:** The mean percentage of histamine receptor bearing cells

Table 1. Detection of histamine receptors on bovine PBL using histamine-rabbit serum albumin conjugate coupled Sepharose beads

Beads treatment	Percentages of beads <sup>(c)</sup>		
	0-10 cells/bead	11-20 cells/bead	>20 cells/bead
H-RSA <sup>(a)</sup>	67.3±3.9	19.0±3.7	14.0±2.2
RSA-ECDI <sup>(b)</sup>	97.0±0.4	3.0±0.4	0
RSA <sup>(c)</sup>	100	0	0
PBS <sup>(d)</sup>	100	0	0

- a) H-RSA: Histamine-rabbit serum albumin conjugate.  
 b) RSA-ECDI: Rabbit serum albumin-ECDI conjugate.  
 c) RSA: Rabbit serum albumin alone.  
 d) PBS: Phosphate buffered saline.  
 e) Mean±standard error of the results from 3 healthy cattle. 100 beads were examined for cells from each animal for each treatment.

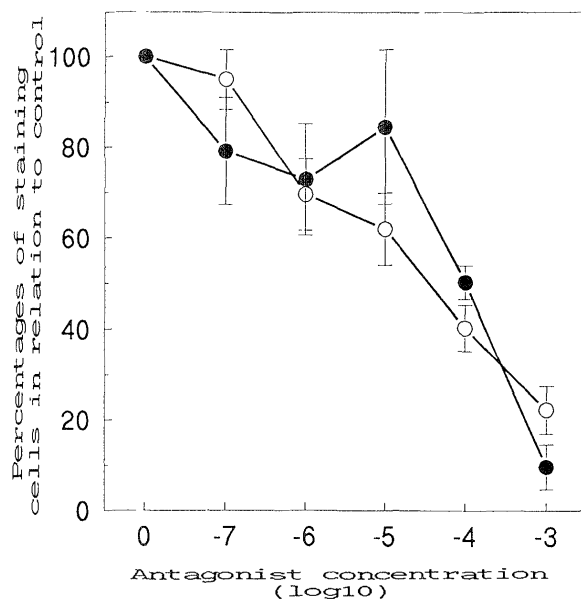


Fig. 1. Blocking of histamine receptors on bovine PBL. Immunofluorescence was used to quantify PBL bearing histamine receptors in the presence of H1 or H2 antagonists. Results are the number of fluorescing cells expressed as a percentage of the number of fluorescing cells in control PBL without antagonists. Results are expressed as mean ± standard error of results from 3 healthy cattle. The open circles represent results obtained using H1 antagonist diphenhydramine and the closed circles represent results obtained using H2 antagonist cimetidine. Concentrations of antagonists are expressed as log<sub>10</sub> (M).

in the B cell and T cell portions of PBL from three healthy cattle are shown in Table 2. More than half (52.8%) of B cells from the peripheral circulation had histamine receptors, while 9.3% of peripheral T cells possessed the receptors. Both H1 and H2 antagonists (10<sup>-4</sup> M) inhibited the detection of histamine receptors on B and T cells to a similar degree.

**Suppressive effect of histamine on responses of bovine PBL to PHA:** Addition of histamine in the concentration range 10<sup>-6</sup> to 10<sup>-3</sup> M suppressed the blastogenic response of bovine PBL to PHA in a dose-dependent manner, whereas 10<sup>-7</sup> M histamine had no detectable effect (Fig.

Table 2. Histamine receptor bearing lymphocytes in B and T cell fraction of bovine PBL using indirect immunofluorescence method

Cell fraction	Percentages of histamine receptor positive cells <sup>(a)</sup>		
	No antagonists	With H1 antagonist <sup>(b)</sup>	With H2 antagonist <sup>(c)</sup>
B <sup>(d)</sup>	52.8±7.2	19.4±1.7 (63%) <sup>(e)</sup>	22.0±8.5 (58%)
T <sup>(e)</sup>	9.3±2.5	3.1±0.5 (66%)	3.9±0.2 (58%)

- a) Mean±standard error of results from 3 cattle.  
 b) 10<sup>-4</sup>M diphenhydramine.  
 c) 10<sup>-4</sup>M cimetidine.  
 d) Surface membrane immunoglobulin positive cells were identified as B cells.  
 e) Rosette forming cells with AET treated sheep red blood cells were designated as T cells.  
 f) ( ): Percentage reduction.

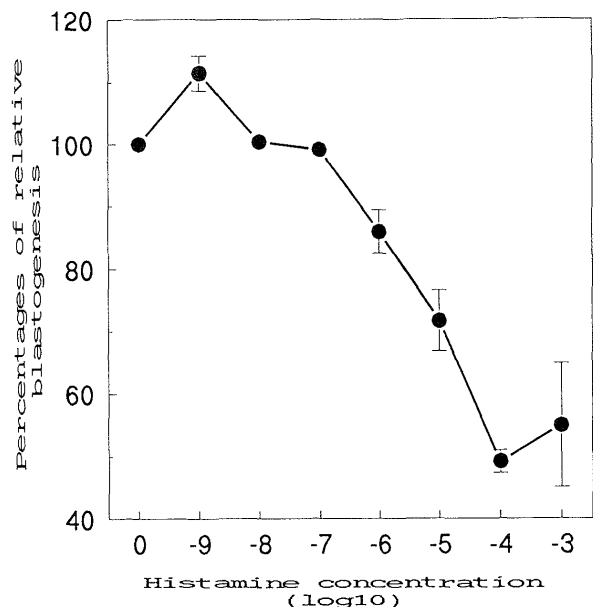


Fig. 2. Suppressive effect of histamine on the response of bovine PBL to an optimal concentration of PHA. Results are expressed as mean ± standard error of lymphocyte blastogenesis expressed as a percentage of controls cultured in the absence of histamine. Results were obtained from 3 healthy cattle. Concentrations of histamine are expressed as log<sub>10</sub> (M).

2). Preliminary experiments showed that higher concentration ( $> 10^{-2}$  M) of histamine killed cells. The suppression of PHA-induced mitogenesis caused by  $10^{-5}$  M histamine was reduced by the H2 receptor antagonist cimetidine at the same concentration, whereas the H1 receptor antagonist diphenhydramine had no detectable effect (Table 3). Neither antagonist had any effect on histamine-induced suppression at lower concentrations. As preliminary experiments showed that higher concentrations ( $10^{-3}$  and  $10^{-4}$  M) of these antagonists suppressed the lymphocyte blastogenesis themselves, it was not possible to test their ability to reverse histamine-mediated suppression of lymphocytes blastogenesis at high concentration.

#### DISCUSSION

Histamine modulates immunological responses in some animal species, suppressing mitogen and antigen induced lymphoproliferation, lymphokine production and immunoglobulin synthesis via histamine receptors on lymphocytes [1, 3]. An immunoregulatory role for histamine has been proposed for a number of diseases in humans and animals, yet until now there has been no information available on histamine receptors on bovine lymphocytes.

We used three different methods to detect histamine receptors on PBL. The RBC rosetting technique has been commonly used to detect histamine receptors on lymphocytes from humans and other animals. Using this method, we found that 10.8% of PBL from bovine animals possessed histamine receptors. This value for cattle is lower than that obtained for mice (22%) [8], Guinea-pigs (7 to 24%) [6], dogs (17.9%) [11] and humans (23.5%) [20].

The method for detecting histamine receptors using H-RSA conjugate-coupled Sepharose beads has been used previously for humans [22] and Jirds [17]. The fact that Sepharose beads carrying H-RSA bound larger numbers of lymphocytes than did beads carrying RSA alone is qualitative evidence that at least some lymphocytes have

histamine receptors. It does not however allow quantitative assessment of the percentage of lymphocytes carrying these receptors.

The third method used in these studies was an immunofluorescence method. The results of the specificity tests indicated that this method was quite specific and that we could detect histamine receptors on bovine PBL. An advantage of the immunofluorescence method over the more commonly used rosetting techniques is that the subpopulation of histamine receptor bearing lymphocytes can be easily double stained to concurrently identify other cell markers. Another advantage is that it is a more stable and more easily quantifiable method. In the present study we were able to detect both histamine receptors and surface membrane immunoglobulin concurrently. The results showed that PBL, and the B cell and T cell fractions of PBL contained 18.4, 52.8 and 9.3% histamine receptor bearing cells, respectively. Saxon *et al.* [20] found that human B cells had 35.0% and T cells 12.4% histamine receptor positive cells. Although their works used the rosetting technique, our findings with bovine B cells were higher than their results and T cells were similar.

Blocking tests using the H1 antagonist diphenhydramine and H2 antagonist cimetidine suggested that bovine PBL have both H1 and H2 receptors on their surface. However we could not determine whether some cells bore both H1 and H2 receptors, or whether each type of receptor was associated with a different population of cells. We estimated that the concentration of histamine (as the histamine-rabbit serum albumin conjugate), used in the immunofluorescence test was approximately  $10^{-4}$  M. The antagonists used at  $10^{-3}$  and  $10^{-4}$  M inhibited the detection of histamine receptors by greater than 50% and it seemed that both antagonists affected histamine binding to a similar extent.

Addition of histamine to cultures of PBL in the concentration ranges  $10^{-6}$  to  $10^{-3}$  M suppressed the response of PBL to PHA, although  $10^{-7}$  and  $10^{-8}$  M histamine had no detectable effect. Responses by normal human PBL were shown to be suppressed by histamine at very low concentrations,  $10^{-9}$  M [16] or  $10^{-8}$  M [1]. In contrast, blastogenesis of PBL from dogs is enhanced by histamine at concentration of  $10^{-4}$  and  $10^{-6}$  M [23]. Clearly lymphocytes from different animal species have different sensitivity and responses to histamine.

Histamine is believed by some authors to enhance lymphocyte mitogenic responses via the histamine H1 receptors and to suppress them via the H2 receptors in humans [1, 2] and dogs [23]. The suppression of mitogenesis induced by  $10^{-5}$  M histamine was reduced marginally by the H2 receptor antagonist cimetidine, while there was no effect with the H1 receptor antagonist diphenhydramine. Both antagonists used at  $10^{-6}$  M and  $10^{-7}$  M had no detectable effect on histamine-induced suppression of mitogenesis. Unfortunately concentrations of  $10^{-4}$  M or greater of the antagonists themselves suppressed lymphocytes blastogenesis. Therefore, we cannot be certain that higher concentrations of the H1 receptor antagonists may

Table 3. Effect of H1 and H2 antagonists on the histamine induced suppression of bovine PBL response to PHA

Concentration	Percentages suppression of PHA response <sup>a)</sup>		
	Histamine without antagonist	Histamine with H1 antagonist <sup>b)</sup>	Histamine with H2 antagonist <sup>c)</sup>
$10^{-5}$ M	28.4±4.9	29.7±3.3	13.8±0.6
$10^{-6}$ M	14.1±3.5	13.5±3.3	11.2±0.8
$10^{-7}$ M	0.9±1.4	4.0±3.8	2.7±3.2

a) Cells were cultured with PHA and histamine with and without either the H1 or H2 antagonists at the same concentration as histamine. Suppression is recorded as the percent reduction in the mitogenic response obtained by culturing cells in PHA alone. Data are shown as mean±standard error of results from 3 healthy cattle.

b) Diphenhydramine.

c) Cimetidine.

not have effects on histamine-induced suppression of mitogenesis.

Sansoni *et al.* [19] found that the histamine induced suppression of lymphocyte function in humans was due to effects exerted by the suppressor T cell subpopulation. Although we did not examine which subpopulation of bovine PBL was concerned with histamine-induced suppression, it is possible that the effects were mediated by suppressor T cells. Interestingly, more than half of the peripheral B cells bore surface histamine receptors. The function of the histamine receptors on B cells is still unknown.

**ACKNOWLEDGEMENTS.** We thank Mr. R. D. Pearson for excellent technical assistance. This work was supported by a grant from Japan International Cooperation Agency.

#### REFERENCES

1. Badger, A. M., Young, J., and Poste, G. 1983. Inhibition of phytohaemagglutinin-induced proliferation of human peripheral blood lymphocytes by histamine and histamine H1 and H2 agonists. *Clin. Exp. Immunol.* 51: 178-184.
2. Beaulieu, L., Beaduoine, J., Jobin, M., and Herbert, J. 1986. Effects of H1 and H2 receptor agonists on nonspecific proliferative response of human peripheral blood lymphocytes. *Int. Archs. Allergy Appl. Immunol.* 79: 249-252.
3. Beer, D. J. and Rocklin, R. E. 1984. Histamine-induced suppressor-cell activity. *J. Allergy Clin. Immunol.* 73: 439-452.
4. Brostoff, J., Pack, S., and Lydyard, P. M. 1980. Histamine suppression of lymphocyte activation. *Clin. Exp. Immunol.* 39: 739-745.
5. De Cock, W., De Cree, J., and Verhaegen, H. 1978. Histamine receptor-bearing T lymphocytes in patients with allergy, autoimmune disease, or recurrent infection. *Clin. Immunol. Immunopathol.* 11: 1-5.
6. Diaz, P., Jones, D. G., and Kay, A. B. 1979. Histamine receptors on guinea-pig alveolar macrophages: chemical specificity and the effects of H1- and H2-receptor agonists and antagonists. *Clin. Exp. Immunol.* 35: 462-469.
7. Hofstetter, M., Fasano, M. B., and Ottesen, E. A. 1983. Modulation of the host response in human schistosomiasis. IV. Parasite antigen induces release of histamine that inhibits lymphocyte responsiveness *in vitro*. *J. Immunol.* 130: 1376-1380.
8. Kedar, E. and Bonavida, B. 1974. Histamine receptor-bearing leukocytes (HRL). I. Detection of histamine receptor-bearing cells by rosette formation with histamine-coated erythrocytes. *J. Immunol.* 113: 1544-1552.
9. Lammie, P. J. and Katz, S. P. 1984. Immunoregulation in experimental filariasis. III. Demonstration and characterization of antigen-specific suppressor cells in the spleen of *Brugia pahangi*-infected jirds. *Immunology* 52: 211-219.
10. Mosmann, T. 1983. Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. *J. Immunol. Methods* 65: 55-63.
11. Ono, K., Mizutani, E., Inokuma, H., Nagao, K., Hasegawa, A., and Tomoda, I. 1987. Histamine receptor bearing lymphocytes in dogs. *Jpn. J. Vet. Sci.* 49: 897-898.
12. Outteridge, P. M. and Dufty, J. H. 1981. Surface markers for characterization of bovine blood lymphocyte populations and changes in these from birth to maturity. *Res. Vet. Sci.* 31: 315-322.
13. Paul, P. S., Senogles, D. R., Muscoplat, C. C., and Johanson, D. W. 1979. Enumeration of T cells, B cells and monocytes in the peripheral blood of normal and lymphocytotic cattle. *Clin. Exp. Immunol.* 35: 306-316.
14. Rocklin, R. E. 1976. Modulation of cellular-immune responses *in vivo* and *in vitro* by histamine receptor-bearing lymphocytes. *J. Clin. Invest.* 57: 1051-1058.
15. Rocklin, R. E. 1977. Histamine-induced suppressor factor (HSF): effect on migration inhibitory factor (MIF) production and proliferation. *J. Immunol.* 118: 1734-1738.
16. Rocklin, R. E., Breard, J., Gupta, S., Good, R. A., and Melmon, K. L. 1980. Characterization of the human blood lymphocytes that produce a histamine-induced suppressor factor (HSF). *Cell. Immunol.* 51: 226-237.
17. Ruff, A. J., Leiva, L. E., Lammie, P. J., and Katz, S. P. 1988. Failure of the H2 antagonist cimetidine to reverse parasite antigen-specific lymphocyte unresponsiveness in experimental filariasis. *Clin. Exp. Immunol.* 74: 26-31.
18. Sahasrabudhe, D. M., McCune, C. S., O'Donnell, R. W., and Henshaw, E. C. 1987. Inhibition of suppressor T lymphocytes (Ts) by cimetidine. *J. Immunol.* 138: 2760-2763.
19. Sansoni, P., Silverman, E. D., Khan, M. M., Melmon, K. L., and Engleman, E. G. 1985. Immunoregulatory T cells in man. Histamine-induced suppressor T cells are derived from a Leu 2+ (T8+) subpopulation distinct from that which gives rise to cytotoxic T cells. *J. Clin. Invest.* 75: 650-656.
20. Saxon, A., Morledge, V. D., and Bonavida, B. 1977. Histamine-receptor leukocytes (HRL). Organ and lymphoid subpopulation distribution in man. *Clin. Exp. Immunol.* 28: 394-399.
21. Smart, L. M. and Kay, A. B. 1981. Histamine receptors on human peripheral blood leucocytes. *Clin. Exp. Immunol.* 44: 581-586.
22. Weinstein, Y., Melmon, K. L., Bourne, H. R., and Sela, M. 1973. Specific leukocyte receptors for small endogenous hormones. Detection by cell binding to insolubilized hormone preparations. *J. Clin. Invest.* 52: 1349-1361.
23. Wilkie, J. S. N. 1991. *In vitro* lymphocytes stimulation by Concanavalin A and with histamine as a co-mitogen in dogs with atopic dermatitis. *Vet. Immunol. Immunopathol.* 28: 67-80.
24. Zarling, J. M., Berman, C., and Raich, P. C. 1980. Depressed cytotoxic T-cell responses in previously treated Hodgkin's and non-Hodgkin's lymphoma patients. Evidence for histamine receptor-bearing suppressor cells. *Cancer Immunol. Immunother.* 7: 243-249.